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Tagging the juvenile locus in soybean [*Glycine max* (L.) Merr.] with molecular markers

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Summary

Soybean cultivars carrying the 'long juvenile trait' show a delayed flowering response under short day conditions. The incorporation of this character into genotypes of agronomic interest may allow a broader range of sowing dates and latitudes for a single cultivar adaptation. The objective of this work was to identify molecular markers linked to the juvenile locus in soybean. Experiments were carried out using two pairs of near isogenic lines (NILs) differing in the presence of the long juvenile trait, and RAPD markers. Four hundred primers were first screened to find polymorphism associated with the trait. Additional differences between NILs were sought by digesting the genomic DNA with five restriction enzymes. Polymorphic fragments detected between NILs were tested for linkage to the juvenile locus in the corresponding F_2 segregating populations. Marker bc357-*Hae*III was linked (χ^2L = 46.316) to the juvenile locus with an estimated recombination frequency of 0.13 $\pm$ 0.03 in one of the genetic backgrounds studied. The fragment was cloned, sequenced and converted into a SCAR marker. Moreover, bc357-*Hae*III was used as RFLP probe. Both, SCAR and RFLP generated markers linked to the juvenile locus in the two genetic backgrounds analysed. Results presented in this work can be utilised for both, the localisation of the gene associated with the character and for tagging the juvenile trait in soybean breeding programs.

Abbreviations: CJ – conventional juvenile phenotype; CTAB – cetyltrimethyl ammonium bromide; LJ – long juvenile phenotype; MAS – marker assisted selection; NILs – near isogenic lines; NIPs – near isogenic pairs; PCR – polymerase chain reaction; RAPD – random amplified polymorphic DNA; RFLP – restriction fragment length polymorphism; SCAR – sequence characterised amplified region; TAE – tris-acetate / ethylendiaminetetra-acetic acid; TE – tris-ethylendiaminetetra-acetic acid

Introduction

Soybean is a short-day plant with a quantitative response to photoperiod (Garner & Allard, 1920). Late sowing dates and/or low latitudes cause premature flowering induction that reduces the length of the vegetative period and restricts the development of leaf area and yield (Criswell & Hume, 1972; Inouye et al., 1979). Consequently, the identification of plants with an extended vegetative period under highly inductive conditions (i.e. short photoperiods and/or high temperatures) is of great interest for breeding. During the last 30 years, several genotypes with this characteristic were identified (Myasaka et al., 1970; Hartwig & Kiihl, 1979; Kiihl & Garcia, 1989). Among them, introduction P.I. 159925 from Peru was first described as 'delayed flowering under short day' (Hartwig & Kiihl, 1979). Afterwards, the term 'long juvenile' (LJ) was proposed for differentiating the genotypes carrying the trait from the conventional juvenile (CJ) plants (Hinson, 1989). Differences of about 10–20 days in the mean flowering date were reported between several near isogenic lines (NILs) that differ for the character

(Sinclair & Hinson, 1992; Cairo et al., 1999). Genetic analysis of segregating populations showed that a single recessive gene controls the LJ trait (Hartwig & Kiihl, 1979; Hinson, 1989; Ray et al. 1995). The symbols *J/j* have been assigned for the alleles conditioning this flowering response, with *jj* the genotype responsible for the LJ phenotype (Ray et al., 1995). The incorporation of the LJ trait into genotypes of agronomic value would increase both the range of latitude and sowing dates adaptation for a single soybean cultivar (Hartwig & Kiihl, 1979).

Traditionally, phenological evaluation of flowering date has been used for identifying plants expressing the long juvenile trait. Differences in the mean flowering date between LJ and CJ plants are particularly clear under short days, making the selection for this character highly dependent of the photoperiodic conditions. Additionally, the expression of the *j* allele is influenced by the genetic background in which it occurs, and by non-photoperiodic environmental effects (Sinclair & Hinson, 1992).

The development of molecular marker technologies enabled the identification of markers linked to traits of interest and the construction of detailed genetic maps of the species (Shoemaker et al., 1996). Particularly, the random amplified polymorphic DNA (RAPD) markers (Williams et al., 1990) have gained wide spread acceptance in basic and applied research (Tingey & del Tufo, 1993). RAPD molecular markers linked to qualitative and quantitative traits in soybean such as resistance to *Phytophtora sojae* gene *Rps1-K* (Kasuga et al., 1997), to sudden death syndrome and cyst nematode Race 3 (Chang et al., 1997) were reported. Several approaches have also been proposed to improve RAPD utility in marker assisted selection (MAS). RAPD markers can be converted into sequence characterised amplified regions (SCAR) by designing a pair of PCR primers specific to the individual RAPD polymorphisms (Paran & Michelmore, 1993). SCARs are less sensitive to reaction conditions, detect a single locus and potentially can be converted into co-dominant markers. A second procedure directly employs the RAPD fragments as RFLP probes (Schulz et al., 1994; Wikie et al., 1993).

One of the most efficient strategies to find markers linked to genes controlling qualitative traits is based on the use of near-isogenic lines (NILs). Markers that are polymorphic between NILs have a high probability of being linked to the gene/s of interest (Diers et al., 1992). In soybean, the *r* and *Lf1* loci were mapped using this method (Muehlbauer et al., 1991).

The objective of this work was the identification of molecular markers linked to the juvenile locus in soybean for assisting the introgression of the character into agronomic important genotypes and tagging the genomic region related to the trait.

Materials and methods

Plant material

Two pairs of near isogenic lines (near isogenic pairs: NIP 1 and NIP 2) of soybean [*Glycine max* (L.) Merr.] differing in the presence of the long juvenile trait were used (Table 1). Both NIPs were developed by Hinson (1989) following a method of repetitive heterozygote selection. Briefly, isoline development began with F_2 populations derived from crosses between CJ and LJ lines. A portion of the early flowering (CJ) plants in the F_2 was known to be heterozygous for the LJ trait. Early flowering F_2 plants were selected and propagated in rows in the F_3. Thereafter, early flowering plants were repeatedly selected from rows segregating for the long juvenile trait until F_6. Plants with CJ and LJ flowering type were selected in the F_7, selfed and checked for homozygocity in the F_8. Thus, lines with CJ and LJ flowering type, derived from a single heterozygous F_6 plant, were established. Each pair obtained in this manner was expected to have $\approx$ 97% of their genes in common and are therefore nearly isogenic (Ray et al., 1995).

Crosses between CJ and LJ lines within each pair (NIP 1 and NIP 2) were carried out to obtain the corresponding F_1. F_2 individuals were classified as having the long juvenile (*jj*) or conventional juvenile (*J_*) trait by comparing the flowering date of each plant to the flowering date of the parental genotypes. Date of flowering was established as the first appearance of an open flower in the main stem and was recorded daily. CJ F_2 individuals were discriminated between homozygous and heterozygous by progeny testing a sample of 6–12 plants of F_3 individuals. The goodness of fit between the observed and expected segregation ratios was determined by a chi square test.

All experiments were conducted at the Laboratorio de Fisiología Vegetal, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Argentina (Lat. 33° 01' S). Plants were grown in pots maintained in a well-watered state with irrigation. Crosses were made in the summer of 1997–1998. F_1 seeds were sown in a greenhouse (26 $\pm$ 2 °C) in June 1998. Natural photoperiod

Table 1. Near-isogenic lines (NILs) used to identify molecular markers linked to the juvenile locus in soybean

Pair	Lineage	NILs[a]	Phenotype designation
NIP 1	Kirby × (Foster (3) × D77-12480[b])	F85-5881	LJ
		F85-592	CJ
NIP 2	Kirby × (Forrest (3) × D77-12480[b])	F85-84932	LJ
		F85-8489	CJ

[a] Lines were obtained by Hinson (1989).
[b] D77-12480 is a selection from Tracy × (Hill × PI 159925), with the latest carrying the LJ trait.

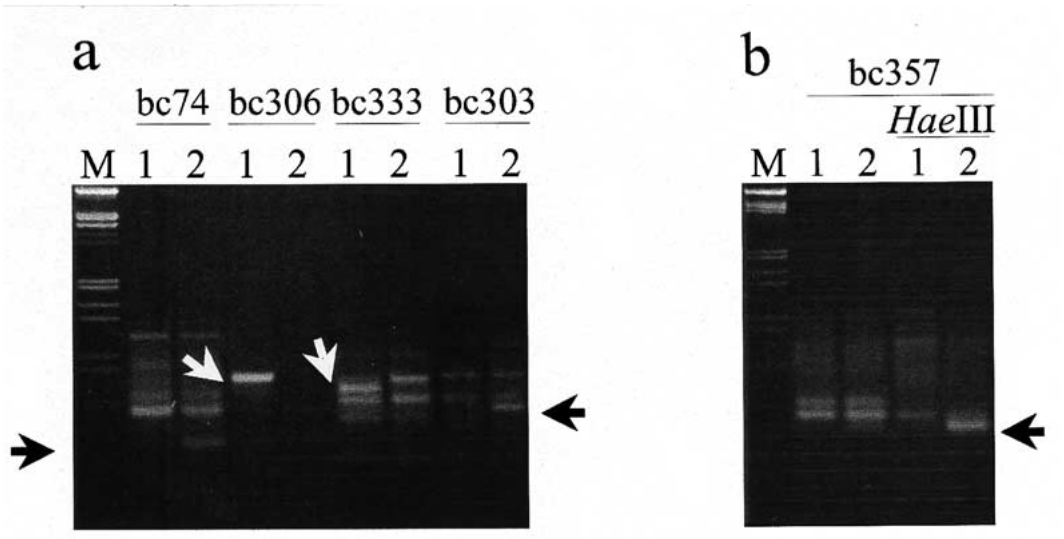

Figure 1. Detection of RAPD polymorphic fragments between NILs. a) Amplification of DNA from NIP 1 (1 = LJ, 2 = CJ) with four UBC primers. b) Amplification of undigested and digested (*Hae*III) DNA from NIP 2 (1 = LJ, 2 = CJ) with primer bc357. Arrows indicate the polymorphic fragments considered. M: molecular weight standards (Lambda DNA /*Eco*RI + *Hin*dIII Markers).

was extended to 16 h with incandescent lamps during the first 20 days from emergence. Afterward, plants were shifted to natural photoperiod (11 h 04 min on average). Four F_2 populations 1a, 1b and 2a, 2b, derived from NIP 1 and NIP 2, respectively, were used. Population 1a was grown in April-May 1999 (average photoperiod 11 h 23 min). Population 1b was grown in June-July 2000 under extended photoperiod (as described above) and then shifted to natural photoperiod (10 h 59 min on average). Population 2a was grown in November-December 1998 under 10 h natural light photoperiod, by daily moving the plants into a dark room (26 ± 2 °C) at 18:00 h and returning them to natural light at 08:00 h. Population 2b was grown in September-November 2000 under natural photoperiod (13 h 03 min on average).

DNA extraction

DNA extraction was performed according to Shagai-Maroof et al. (1984) with the following modifications. Plant tissue (0.2 g of leaves) was frozen in liquid nitrogen, powdered with a mortar and pestle and incubated in 1 ml of CTAB extraction buffer for 1 hour at 65 °C. Samples were extracted twice with one volume of chloroform. The top phase was gently removed and incubated in 1 ml of 95% ethanol at –20 °C for at least twenty minutes. DNA was picked up using a Pasteur pipette, washed in 75% ethanol / sodium acetate 0.2 M and air-dried. The DNA was re-suspended in TE buffer pH 8 and treated with RNAase A (0.25 mg/ml) for 90 minutes at 37 °C. Finally, samples were ethanol precipitated and dissolved in 100 μl of sterile water.

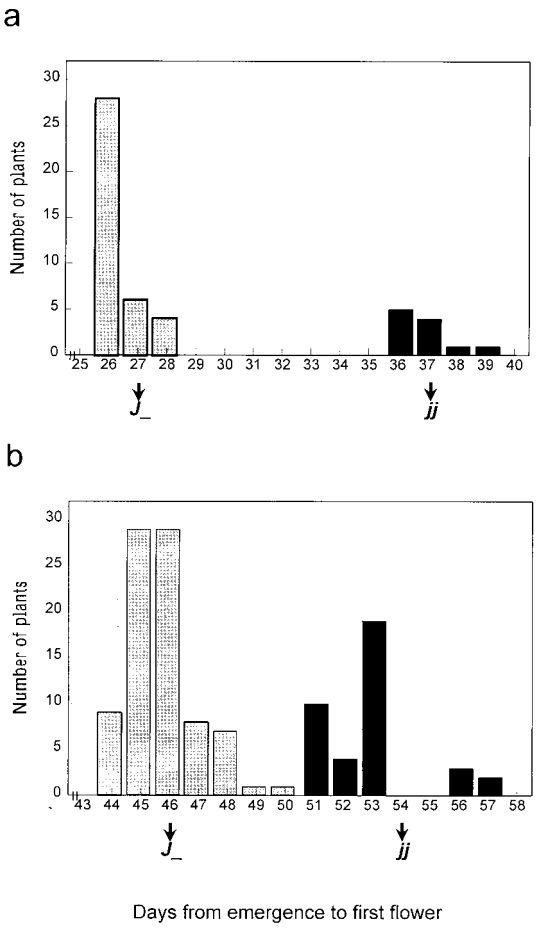

Figure 2. Flowering date distribution of F_2 populations. a) Population 1a was obtained from a cross between lines within NIP 1. b) Population 2a was obtained from a cross between lines within NIP 2. Grey bars represent F_2 individuals classified as having a CJ flowering response (*J_*). Black bars represents F_2 individuals classified as having a LJ type response (*jj*). Arrows indicate the flowering day of the parental genotypes.

RAPD analysis

RAPD analyses were carried out employing LJ and CJ individuals from the two NIPs and 400 decamer primers of arbitrary nucleotide sequence supplied by the Biotechnology Laboratory of the University of British Columbia, Vancouver, Canada (UBC), sets No. 1, 3, 4 and 5 (each set contains 100 primers). Amplifications were carried out in 25 μl of total volume reactions containing: 1 ng/μl DNA, 1 × *Taq* buffer (Promega), 15 μM of each dNTP, 1.5 mM $MgCl_2$, 1.25 U *Taq* enzyme (Promega) and 0.36 μM of primer. After all primers had been screened a search for additional polymorphisms was initiated by using oligonucleotides of sets 4 and 5 in reactions containing the genomic DNA digested with the restriction enzymes *Alu*I, *Hae*III, *Hin*dIII, *Eco*RI or *Taq*I (Promega). Digestions were carried out following the supplier's recommendations (Promega). Amplifications were performed in a MJ Research, Inc. PTC-100 Programmable Thermal Controller. Cycles began with 2 min at 94 °C followed by 45 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C. A final incubation of 5 min at 72 °C was also included. Reaction products were electrophoresed in 2–2.5% agarose gel / 1 × TAE buffer at 60 mA for 3 h, stained with ethidium bromide and photographed under UV light with a Polaroid 667 film. Markers were named according to the RAPD primer used in the amplification followed by the molecular weight of the considered fragment in base pairs (bp) or the enzyme employed for the digestion of the DNA. Amplification reactions that detected polymorphism between isolines were carried out independently on three or more separate occasions to assure reproducibility.

RAPD marker cloning and SCAR development

A slice of low melting point agarose gel, with the desired RAPD fragment, was incubated at 65 °C in 300 μl of TE buffer pH 8.0 supplemented with 100 μl of ammonium acetate 3 M pH 5.2 for 10 min. DNA was extracted twice with pre-warmed phenol (65 °C) and once with chloroform. Samples were ethanol precipitated overnight at –20 °C, centrifuged (30 min, 12,000 g), washed thoroughly in 80% ethanol, air dried and dissolved in 20 μl of sterile water. The purified fragment was cloned in a pGEM-T vector according to the manufacturer instructions (Promega). The insert was sequenced at the University of Maine DNA Sequencing Facility and a pair of oligonucleotides was designed to each end of the sequence. Optimal conditions for SCAR amplification were empirically determined and included: 1 ng/μl DNA, 1 × *Taq* buffer (Promega), 50 μM of each dNTP, 1.5 mM $MgCl_2$, 1.25 U *Taq* (Promega) and 0.5 μM of both upper and lower primers in 25 μl of final volume. Amplification cycles began with 1 min at 95 °C followed by 35 cycles of 1 min at 95°C, 1 min at 52 °C and 1 min at 72 °C. A final incubation of 5 min at 72 °C was included. Amplified products were resolved as described above.

RFLP experiments

Hybridizations were carried out using DNA from NIPs digested with the enzymes *Hae*III, *Hin*dIII and *Eco*RI.

Table 2. Segregation ratios of the juvenile locus and molecular markers that were polymorphic between NILs

Locus	F_2 Population[a]	Expected ratio	Observed ratio	*n*	χ^2	P[d]
juvenile	1a	1:2:1[b]	13:25:11	49	0.184	0.912
bc74-331	1a	3:1[c]	34:15	49	0.823	0.364
bc303-560	1a	3:1[c]	37:12	49	0.006	0.938
bc306-710	1a	3:1[c]	33:16	49	1.530	0.216
bc333-634	1a	3:1[c]	38:11	49	0.169	0.681
scar357-220	1a	3:1[c]	37:12	49	0.006	0.938
juvenile	1b	3:1[c]	29:9	38	0.012	0.913
p357	1b	1:2:1[c]	13:17:8	38	1.629	0.443
juvenile	2a	3:1[c]	84:28	112	0.000	1.000
bc357-*Hae*III	2a	3:1[c]	84:27	111	0.009	0.924
scar357-260	2a	3:1[c]	37:14	51	0.163	0.687
juvenile	2b	1:2:1[b]	11:26:9	46	0.956	0.620
p357	2b	1:2:1[c]	5:31:10	46	5.826	0.054

[a] F_2 populations 1a-b: obtained from crosses LJ × CJ within NIP1. F_2 populations 2a-b: obtained from crosses LJ × CJ within NIP2.
[b] CJ F_2 individuals were discriminated between homozygous and heterozygous by testing the phenotype of a sample of 6–12 F_3 plants from each CJ F_2.
[c] Tested on the F_2 population.
[d] Probability of a greater value of χ^2 by chance alone.

Twenty micrograms of digested DNA were loaded in 1% agarose gel / 1 × TAE buffer, electrophoresed at 60 mA and blotted onto nylon membranes (Hybon N, Amersham) using 10X SSC. DNA was fixed to the membrane by 3 min exposition to UV-light in a high performance ultraviolet transilluminator (UVP) and by baking at 80 °C for 2 h. Cloned bc357-*Hae*III RAPD fragment was used as an RFLP probe. Labelling reaction was performed in 100 μl of total volume reactions including: 0.1 ng/μl plasmid DNA, 1 × *Taq* buffer (Promega), 50 μM of each dNTP, 2mM $MgCl_2$, 0.2 μM of each forward and reverse M13 primers, 5% dig-dUTP (digoxigenin 11-dUTP, Boehringer-Mannheim), 15% glycerol and 1 U *Taq* enzyme (Promega) and the following amplification parameters: 25 cycles of 1 min at 94 °C, 2 min at 55 °C and 2 min at 72 °C. Hybridization and detection procedures were performed according to the DIG Luminescent Detection Kit (Boehringer-Mannheim), using CSPD® (disodium 3-(4-methoxyspiro1, 2-dioxetane-3, 2'- (5'-chloro) tricyclo [3.3.1.1^{3,7}] decan-4-yl) phenyl phosphate) (Boehringer-Mannheim) as a chemiluminescent substrate. Filters were exposed 4–12 h to X-Omat films (Kodak).

Linkage analysis

Markers found to be polymorphic between NILs were tested for linkage to the juvenile locus in F_2 segregating populations. The data were first examined for single-factor segregation and linkage using a Chi square test. Recombination frequencies were estimated by the method of maximum likelihood (Mather, 1951; Allard, 1956).

Results

RAPD markers analysis

Primer screening on DNA from NIP 1 and NIP 2 showed that the 84% of them amplified 1 to 6 bands, while the rest (16%) displayed either smearing or lack of amplification. On average, 3.5 fragments were generated by each informative primer allowing the evaluation of some 1,176 markers. Most of the informative primers (98.8%) showed a monomorphic pattern between isolines, but 4 of them (1.2%) (bc74, bc303, bc306 and bc333) revealed differences in NIP 1. Primers bc306 and bc333 generated fragments present only in the LJ isoline, while primers bc74 and bc303 generated fragments specific of the CJ isoline (Figure 1a). Since there were no polymorphism in NIP

2, the genomic DNA was digested with five restriction enzymes (see Material and methods) and tested again with 165 primers from UBC sets No. 4 and 5. Only oligonucleotides that produced good amplification patterns in the former experiments were used. This strategy permitted the evaluation of some 2,100 additional RAPD fragments. As a result, a marker of 260 bp, named bc357-*Hae*III, was obtained in NIP 2 when primer bc357 and the enzyme *Hae*III were used (Figure 1b). The polymorphism resulted in the amplification of an extra band presented in the CJ isoline and absent in the LJ isoline.

Segregation and linkage analysis

The four F_2 populations developed (1a-b and 2a-b) segregated for the long juvenile trait in a 3:1 (CJ vs. LJ) expected phenotypic ratio. Results obtained from a progeny test of populations 1a and 2b agreed with the 1:2:1 expected genotypic ratio (Table 2). Populations 1b and 2a were only phenotypically classified (Table 2). Figure 2 shows an example of the overall bimodal distribution of time to first flower observed for populations 1a and 2a, derived from NIP 1 and NIP 2, respectively.

RAPD markers showing polymorphism between NILs were assayed with the corresponding F_2 populations to assess their linkage to the juvenile locus. All markers segregated in a 3:1 ratio expected for a single dominant factor (Table 2). Results of linkage analysis are presented in Table 3. All polymorphic markers in NIP 1 (bc74-331, bc303-560, bc306-710, and bc333-634) segregated independently from the long juvenile locus ($0.062 < \chi^2 L < 4.363$) when tested on population 1a. However, marker bc357-*Hae*III detected in NIP 2 was linked ($\chi^2 L = 46.316$) to the CJ trait, when tested on population 2a with an estimated recombination frequency (r) of 0.13 ± 0.03.

SCAR and RFLP experiments

In order to improve the specificity and reproducibility of marker bc357-*Hae*III, a pair of primers corresponding to both ends of the fragment was designed to convert it into a SCAR marker. Primers included the original decamer used for the RAPD amplification (in bold letter) and additional nucleotides (357-upper: 5' T**AG GCC AAA TG**C AAT AGA CTG T '3 and 357-lower: 5' T**AG GCC AAA TG**T AAG GTA AAC '3). The PCR reaction on DNA from NIP 2 with the scar primers generated a strong band of 260 bp (scar357-260) presented only in the CJ isoline (Figure 3). As expected, scar357–260 showed a similar recombination value with the juvenile locus as did bc357-*Hae*III when it was tested on a sample of population 2a (n = 51) (Table 3). When scar357 was assayed on population 1a, a polymorphic band of 220 bp (scar357-220) that co-segregated with the long juvenile trait was also detected. Scar357-220 showed an estimated $r = 0.19 \pm 0.06$ with the juvenile locus (Table 3).

Hybridization of DNA from NIP 2 with a probe derived from marker bc357-*Hae*III (p357) revealed polymorphisms with all the restriction enzymes tested (not shown). Linkage analysis performed on DNA from population 2b digested with *Eco*RI showed an estimated $r = 0.20 \pm 0.05$ (Table 3). Hybridization results made possible the identification of the homozygous and heterozygous CJ genotypes (Figure 4a). Similar results were obtained when probe p357 was hybridized with DNA from population 1b digested with *Hin*dIII (Table 3, Figure 4b).

Discussion

The introgression of the single recessive gene for the long juvenile trait into soybean cultivars would require reliable methods for an early discrimination of plants carrying the *j* allele. This practice should avoid interferences related to the genetic background in which the gene is expressed and the environmental conditions where the selection is done. Molecular markers could successfully fulfil these requirements because they are free of both environmental and genetic effects. The use of RAPDs markers in combination with NILs allowed the identification of polymorphisms associated with the juvenile locus.

In NIP 1, genetic analyses showed that none of the RAPD polymorphic fragments detected between the NILs were linked to the character (Table 3). Markers bc74-331, bc303-560, bc306-710 and bc333-634 may have detected different genomic regions that remained heterozygous at the F_6, and would have achieved an alternative homozygous constitution by chance during the isoline stabilisation, resulting in polymorphism between LJ and CJ isolines. In NIP 2, a single marker (bc357-*Hae*III) that showed polymorphism between NILs was linked to the juvenile locus (Table 3). When marker bc357-*Hae*III was converted into a SCAR and assayed on a sample of the F_2 population, cosegregation with the character was confirmed and the specificity of the PCR assay was improved. Amplification of DNA from NIP 1 with

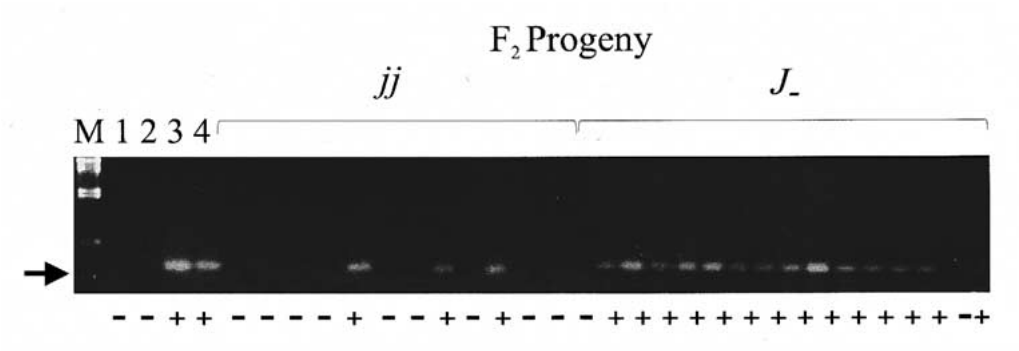

Figure 3. Amplification of DNA from parents of population 2a, F_1 and a sample of the F_2 progeny using 357-upper and 357-lower scar primers. M: molecular weight standards (Lambda DNA /*Hin*dIII Markers). 1: negative control. 2: LJ parent. 3: CJ parent. 4: F_1 individual. *J_* and *jj*: F_2 individuals classified as having a CJ or LJ flowering response, respectively. The arrow indicates the fragment corresponding to marker scar357-260.

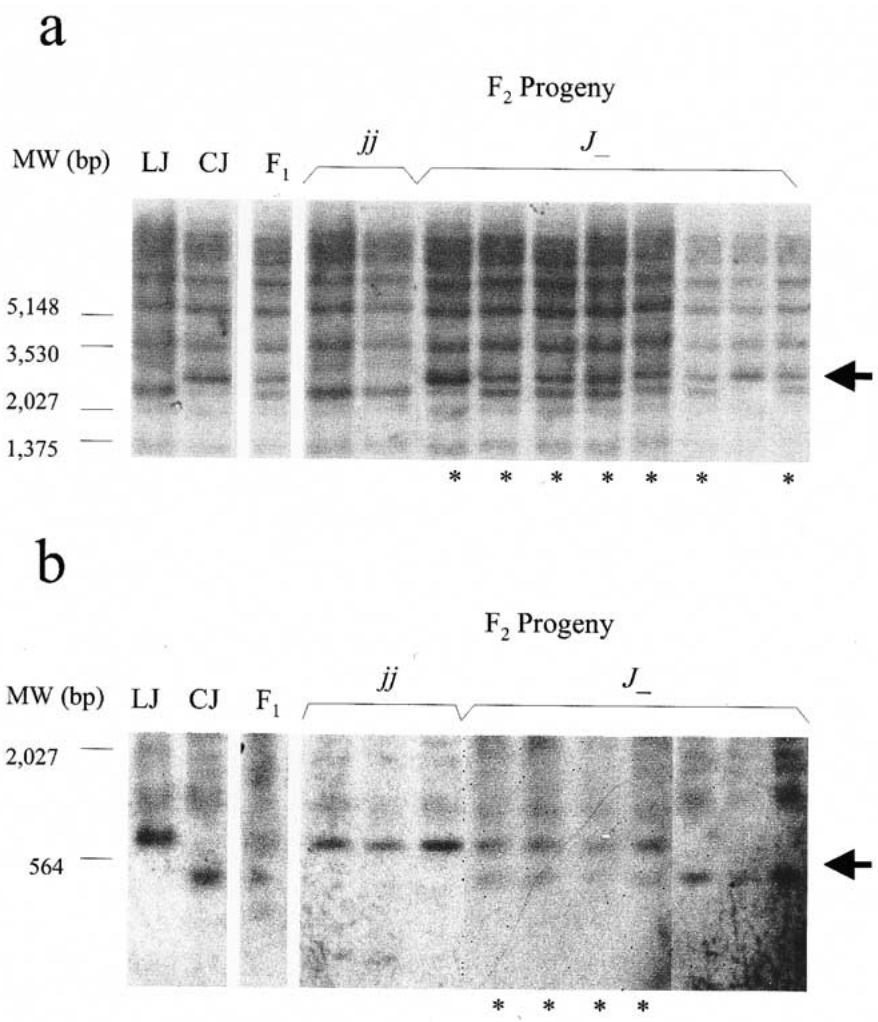

Figure 4. RFLP experiments with probe p357. a) Hybridization of DNA from population 2b digested with *Eco*R1. b) Hybridization of DNA from population 1b digested with *Hind*III. LJ and CJ: parental genotypes, *jj* and *J_*: LJ and CJ F_2 individuals. Arrow indicates markers scored. Asterisks show CJ heterozygous plants. MW: molecular weight standards (Lambda DNA /*Eco*RI + *Hin*dIII Markers, Promega).

Table 3. Linkage data for the molecular markers and the juvenile locus in F_2 soybean populations

F_2[a]	Loci	Genotypic classes[b]													*n*	χ^2L[c]	P[d]	r[e]	SE[f]
		a	b	c	d	e	f	g	h+i	j	k	l	m	n					
1a	bc74-331/ juvenile					9		20		5		4	5	6	49	4.363	0.113	–	–
1a	bc303-560/ juvenile					10		18		9		3	7	2	49	0.388	0.824	–	–
1a	bc306-710/ juvenile					9		17		7		4	8	4	49	0.062	0.970	–	–
1a	bc333-634/ juvenile					10		19		9		3	6	2	49	0.118	0.943	–	–
1a	scar357-220/ juvenile					5		21		11		8	4	0	49	14.210	0.000	0.19	0.06
1b	juvenile/ p357					8		16		5		0	1	8	38	17.973	0.000	0.16	0.06
2a	juvenile/ bc357-*Hae*III	77	7	7	20										111	46.316	0.000	0.13	0.03
2a	juvenile/ scar357-260	34	4	3	10										51	23.121	0.000	0.14	0.05
2b	juvenile/ p357					2	3	8	21	1	2	0	1	8	46	23.131	0.000	0.20	0.05

[a] $F_2$1a-b: obtained from crosses LJ × CJ within NIP1. $F_2$2a-b: obtained from crosses LJ × CJ within NIP2.
[b] Class designation per Allard (1956). a = A_B_, b = A_bb, c = aa B_, d = aabb, e = A_BB / AABB, f = AaBB, g = A_Bb / AABb, h+i = AaBb, j = A_bb / AAbb, k = Aabb, l = aaBB, m = aaBb, n = aabb. A and a: dominant and recessive alleles from the first locus listed, respectively. B and b: dominant and recessive alleles from the second locus listed, respectively.
[c] Linkage chi – square.
[d] Probability of a greater value of χ^2L by chance alone.
[e] Estimates of recombination frequency by the maximum likelihood method.
[f] Standard error of recombination estimates.

SCAR primers also generated a marker linked to the trait in this genetic background (Table 3). Although it was suggested that large differences in linkage could exist between identical markers when tested in different genetic backgrounds (Palmer & Shoemaker, 1987), scar357 showed a similar association with the juvenile locus in the two NIPs analysed.

The use of bc357-*Hae*III as an RFLP probe generated a co-dominant marker that may be used for distinguishing between homozygous (*JJ*) and heterozygous (*Jj*) CJ plants in order to recover the *j* allele from the latest. Moreover, marker p357 can potentially be employed for detecting the linkage group carrying the juvenile locus by assaying it in a soybean mapping population. This procedure will facilitate the identification of new markers linked to the trait.

Results reported in this work demonstrated that markers generated from primer bc357 (bc357-*Hae*III, scar357-260, scar357-220 and p357) were linked to the juvenile locus in two genetic backgrounds of soybean. Although markers available so far are not closely linked to the character, they can be used for an early discrimination of LJ plants in a segregating population, avoiding environmental and genetic effects in the expression of the character. This procedure will improve the overall efficiency of a breeding program aimed at the incorporation of the long juvenile trait into commercial cultivars. Markers reported here can also be employed as a starting point in strategies directed to the isolation of the gene responsible for the character.

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References

Allard, R.W., 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24: 235–278.

Cairo, C.A., E.N. Morandi & T.R. Sinclair, 1999. Analysis of flowering response to photoperiod and temperature in long juvenile soybean isolines. In: H.E. Kauffman, University of Illinois (Ed.), Proceedings of Soybean Research Conference VI. 4–7 August, Chicago, USA, pp. 530.

Chang, S.J.C., T.W. Doubler, V.Y. Kilo, J. Abu-Thredeih, R. Prabhu, V. Freire, R. Suttner, J. Klein, M.E. Schmidt, P.T. Gibson & D.A. Lighfoot, 1997. Association of loci underlying field resistance to soybean sudden death syndrome (SDS) and cyst nematode (SCN) race 3. Crop Sci 37: 965–971.

Criswell, J.C. & D.J. Hume, 1972. Variation in sensitivity to photoperiod among early maturing soybean strains. Crop Sci 12: 657–660.

Diers, B.W., J. Mansur, J. Imsande & R.C. Shoemaker, 1992. Mapping *Phytophthora* resistance loci in soybean with restriction fragment length polimorphism markers. Crop Sci 32: 377–382.

Garner, W.W. & H.A. Allard, 1920. Effect of the relative length of day and night and other factors of environment on growth and reproduction in plants. J Agri Res 18: 553–606.

Hartwig, E.E. & R.A.S. Kiihl, 1979. Identification and utilisation of a delayed flowering character in soybean for short-day conditions. Field Crops Res 2: 145–151.

Hinson, K., 1989. Use of long juvenile trait in cultivar development. In: Pascale, A. (Ed.), Proc. World soybean research conference IV, 5 to 9 March 1989, pp. 983–987. Buenos Aires, Argentina.

Inouye, J., S. Shanmugasundaram & T. Masuyama, 1979. Effects of the temperature and daylength on the flowering of some photoinsensitive soybean varieties. Japan J Trop Agric 22: 167–172.

Kasuga, T, S.S. Salimath, J.R. Shi, M.Gijzen, R.I. Buzzell & M.K. Bhattacharyya, 1997. High-resolution genetic and physical mapping of molecular markers linked to the *Phytophthora* resistance gene Rps1-K in soybean. Mol Plant-Micr Int 10: 1035–1044.

Kiihl, R.A.S. & A. Garcia, 1989. The use of the long-juvenile trait in breeding soybean cultivars. In: Pascale, A. (Ed.), Proc World soybean research conference IV, 5 to 9 March 1989, Buenos Aires, Argentina, pp. 994–1000.

Mather, K., 1951. The Measurement of Linkage in Heredity, 2nd edn., Methuen, London

Miyasaka, S., G. Guimaraes, R.A.S. Kiihl, L.A.C. Lovadini & J.C. Dematte, 1970. Variedades de soja indiferentes ao fotoperiodismo e tolerantes a baixas temperaturas. Bragantia 29: 169–174.

Muehlbauer, G.J., J.E. Staswick, P.E. Spech, G.L. Graef, R.C. Shoemaker & P. Keim, 1991. RFLP mapping using near-isogenic lines in soybean [*Glycine max* (L.) Merr.]. Theor Appl Genet 81: 189–198.

Palmer, R.G. & R.C. Shoemaker, 1987. Linkage map of soybean (*Glycine max* L. Merr.) In: S.J. O'Brien (Ed.), Genetic map 1987, vol. 4, pp. 732–741.

Paran, A. & R. Michelmore, 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85: 985–993.

Ray, J.D., K. Hinson, J.E.B. Mancono & M. Malo, 1995. Genetic control of a long-juvenile trait in soybean. Crop Sci 35: 1001–1006.

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen & R.W. Allard, 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci 81: 8014–8018.

Schulz, B., L. Westphal & G. Wricke, 1994. Linkage groups of isozymes, RFLP and RAPD markers in carrot (*Daucus carota* L. *sativus*). Euphytica 74: 67–76.

Shoemaker, R.C., K.M. Polzin, L.L. Lorenzen & J.E. Specht, 1996. Molecular genetic mapping of soybean. In: D.P.S. Verma & R.C. Shoemaker (Eds.), Soybean: Genetics, Molecular Biology and Biotechnology, pp. 37–56. Centre for Agriculture and Bioscience International.

Sinclair, T.R. & K. Hinson, 1992. Soybean flowering in response to the long juvenile trait. Crop Sci 32: 1242–1248.

Tingey, S.V. & J.P. del Tufo, 1993. Genetic analysis with random amplified polymorphic DNA markers. Plant Physiol 101: 349–352.

Wikie, S.E., P.G. Isaak & R.J. Slater, 1993. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *allium.* Theor Appl Genet 86: 497–504.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski & S.V. Tingley, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl Acids Res 18(22): 6531–6535.